RETENTION OF TIMOLOL IN THE OCULAR TISSUES OF THE RABBIT

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IN THE
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ABSTRACT

The β-adrenergic antagonist timolol has been shown to be an effective agent in decreasing intraocular pressure (IOP) in both experimental animals and humans. The mechanism by which this is achieved, however, is not understood. Many feel that timolol decreases IOP by blocking β-adrenergic receptors in the ciliary processes, leading to a decrease in formation of aqueous humor, while others believe that timolol effects a decrease in IOP by a mechanism other than β-adrenergic blockade. Those who support the latter proposal cite as evidence the fact that timolol has been shown to act as a β-adrenergic antagonist at low concentrations, but that higher concentrations are needed to cause a decrease in IOP.

The purpose of this study was to estimate the retention of timolol in the ocular tissues of the New Zealand White rabbit after topical application. Two different doses of timolol were used; one that was capable of decreasing IOP, and the other incapable of decreasing IOP. If timolol were in fact decreasing IOP by β-adrenergic antagonism, the concentrations of timolol in the ciliary processes after topical application of both concentrations should be close to K_I for timolol binding to the β-adrenergic receptors in this tissue; however, if this were not the mechanism, then the concentrations of timolol in the ciliary processes should be much higher than K_I.

It was demonstrated that the concentration of timolol in the ciliary processes exceeded the concentration needed to effect β-adrenergic blockade by a factor of 10^2 to 10^3, even when the dose of timolol used was incapable of lowering IOP. These findings support the theory that timolol decreases IOP by a mechanism other than β-adrenergic blockade.

Also, in this thesis, the literature concerning the effects of adrenergic agents upon aqueous humor dynamics and IOP is reviewed.

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Dedicated to my parents,

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INTRODUCTION

Drugs influencing the adrenergic receptors of the eye have been used in treating glaucoma since the early 1920s when the Berlin ophthalmologist Hamburger reported that subconjunctival injection of epinephrine caused a significant decrease in intraocular pressure (IOP) in patients with acute and chronic glaucoma.[1,2] Earlier reports by Erdmann,[3] Knapp,[4] and Darier[5] demonstrate the extensive early interest in epinephrine as a treatment for glaucoma. The separation of the adrenergic system into the α and β components by Ahlquist[6] resulted in new attempts to understand the role of the adrenergic system in regulating IOP. In the 1950s, Weekers et al.[7] showed that the β-adrenergic agonist isoproterenol, applied topically to the eye, produced a decrease in IOP comparable to that caused by epinephrine. Since then, considerable effort has been made to understand the mechanism of the adrenergic influences on IOP.

Early studies investigated the role of the adrenergic system in controlling IOP by excising the cervical sympathetic nervous system in rabbits and then monitoring IOP and outflow facility. The earliest studies by Linner and Prijot[8,9] and Lieb et al.[10] showed that preganglionic cervical sympathectomy had no effect on IOP, inflow, or facility of outflow, but that excision of the superior cervical ganglion resulted in a marked decrease in IOP 24 hours after the procedure, followed by a return to normal.

Constant pressure perfusion studies in these animals seemed to show that the outflow facility remained similar to that found in the control eyes. This led to the conclusion that the decrease in IOP after ganglionectomy was the result of a marked decrease in the formation of aqueous humor.

On the other hand, other studies of the ganglionectomy effect[11-18] demonstrated an increase in facility associated with the observed decrease in IOP. Langham and Taylor[11,12] noted that an additional decrease in the IOP could be achieved by the administration of acetazolamide, a carbonic anhydrase inhibitor which decreases the rate of aqueous formation,[19] demonstrating that secretion of aqueous humor had previously been present. These findings suggested that an increase in outflow facility rather than the previously proposed decrease in aqueous humor secretion was the mechanism by which ganglionectomy caused the observed reduction in IOP.

Sears and Barany,[13] by directly monitoring IOP by intracameral cannulation and manometry, documented that the α-adrenergic antagonist dibenamine decreased outflow facility, while ganglionectomy increased outflow facility. They concluded that the decrease in IOP at 24 hours after ganglionectomy was largely due to the release of α-adrenergic neurotransmitters from the degenerating adrenergic nerve endings. This conclusion was supported by the further findings of Barany,[14] Sears and Sherk,[15,16] Rosser and Sears,[17] and Eakins and Eakins,[18] who together demonstrated

that gangionectomy resulted in a loss of norepinephrine from the iris and ciliary body, apparently secondary to the degeneration of adrenergic nerve endings in these tissues. Norepinephrine, an α-adrenergic agonist, caused an increase in outflow facility upon release from the nerve endings, most likely by stimulating α-adrenergic receptors in the trabecular meshwork. The α-adrenergic antagonists dibenamine[13] and phenoxybenzamine[14,16] inhibited the norepinephrine-induced increase in outflow facility. Also, prior depletion of catecholamine stores in the tissue by reserpine or guanethidine prevented the increase in facility after ganglionectomy.[14]

Hendley and Crombie[20] showed that propranolol, a β-adrenergic antagonist, had no effect on the decrease in IOP or the increase in outflow facility in eyes of ganglionectomized animals, while phentolamine, an α-adrenergic antagonist, blocked both effects. This led to the conclusion that α-adrenergic but not β-adrenergic receptors were involved in regulating outflow facility.

In other studies, various α- and β-adrenergic agonists were administered intravenously, intravitreally, subconjunctivally, or topically to rabbits in all possible combinations and at every conceivable dose. Eakins[21] treated rabbits intravitreally with epinephrine, norepinephrine, and isoproterenol. These studies revealed that epinephrine in small doses decreased IOP without affecting outflow facility, while larger doses of epinephrine decreased IOP

and also slightly increased outflow facility. Norepinephrine consistently decreased IOP and increased facility. Isoproterenol usually resulted in a decrease in IOP without changing outflow facility; however, large doses of isoproterenol were observed to increase outflow facility. Eakins concluded that α-adrenergic stimulation resulted in increased outflow facility, that isoproterenol caused a decrease in IOP probably by decreasing aqueous humor formation, and that small doses of epinephrine behaved very similarly to isoproterenol in the rabbit; in other words, like a β-adrenergic agonist.

Further studies by Eakins and Ryan[22] supported the concept that α-adrenergic agonists lower IOP by increasing outflow facility. Phentolamine was shown to decrease significantly the effect of norepinephrine upon IOP and outflow facility. The increase in facility of outflow caused by large doses of isoproterenol was completely inhibited by phentolamine. This supported the earlier conclusion of Butterworth[23] that, at high doses, isoproterenol was capable of stimulating α-adrenergic receptors. Ahlquist[24] has also reported that isoproterenol possesses α-adrenergic stimulatory properties. Work published at the same time by Gnädinger and Barany[25] also demonstrated that isoproterenol led to an increase in outflow facility, and Gnädinger concluded that β-adrenergic stimulation led to increased facility. Sears and Barany[13] also demonstrated that dichloroisoproterenol (DCI), a β-adrenergic antagonist with partial β-adrenergic stimulatory properties, also increased outflow

facility. However, the doses of isoproterenol used by Gnädinger were quite high; higher, in fact, than the doses at which Eakins had earlier reported that isoproterenol apparently possessed mild α-adrenergic agonistic behavior.[22] Because of these conflicting findings, the role of pure β-adrenergic stimulation in increasing outflow facility in rabbits is unclear.

In summary, these studies have demonstrated that, in the rabbit, α-adrenergic stimulation causes an increase in outflow facility and a decrease in IOP, while β-adrenergic stimulation causes a decrease in IOP and may also increase outflow facility. Weekers et al.[7] and Eakins[21] postulated that the β-adrenergic-induced decrease in IOP may have been secondary to a decrease in aqueous humor formation. Other studies in rabbits with adrenergic agonists support this hypothesis that β-adrenergic stimulation most likely caused a decrease in formation of aqueous humor, leading to a decrease in IOP.[26-30]

Langham and Diggs[29] demonstrated that salbutamol, a specific β_2-adrenergic agonist, when applied topically or injected intravitreally in rabbit eyes, led to a decrease in IOP greater than that observed with isoproterenol. Their three studies,[27-29] when considered together, indicate that the α-agonist-induced decrease in IOP is delayed, with minimal pressures achieved at 4 to 6 hours, while the decrease in IOP induced by isoproterenol or salbutamol is of rapid onset, with maximal decreases in IOP noted at 1 to 2 hours.

The fact that the onset of the decrease in IOP is so different for α- and β-adrenergic stimulation supports the conclusion that α- and β-adrenergic stimulation lead to a decrease in IOP via different mechanisms.

The studies of Potter and Rowland[31] again demonstrated the effectiveness of salbutamol in decreasing IOP. Also, the β_2-adrenergic agonists isoetharine, metaproterenol, terbutaline, carbuterol, quinterenol, and sulfonterol all decreased IOP; the maximum decrease occured between $\frac{1}{2}$ and 2 hours after topical application. The selective α-adrenergic agonists norepinephrine and phenylephrine showed a delayed decrease in IOP, with minimal IOP achieved at 4 hours. Rowland and Potter[32] also demonstrated that the β_2-adrenergic agonist reproterol was effective in blunting the water-load-induced increase in IOP in rabbits, while the selective β_1-adrenergic agonist tazolol was not. The results of these studies indicate that stimulation of α-adrenergic receptors leads to a decrease in IOP by increasing facility, while stimulation of β (and more specifically β_2) adrenergic receptors results in a decrease in IOP by decreasing formation of aqueous humor.

The effects of adrenergic stimulation upon IOP and outflow facility in rabbit eyes are summarized in Table 1.

Barany[33] perfused the anterior chamber of vervet monkeys with fluid, constantly monitoring fluid flow rate and pressure, and noted an increase in outflow facility but no change in IOP after topical application of epinephrine.

Bill[34] demonstrated in vervet monkeys that epinephrine caused an increase in IOP at 1 hour that had disappeared by 2 hours, along with a significant decrease in the flow rate of aqueous humor, and an increase in facility. In a later study, Bill[35] demonstrated that isoproterenol increased aqueous humor formation by 30% and facility of outflow by 55%. Both effects were abolished by propranolol.

It is dangerous to generalize the physiology of aqueous humor dynamics in rabbits and monkeys to man. Therefore, the studies that have been performed with human subjects are of particular interest. In man, Weekers et al.[7] demonstrated that topical epinephrine led to a decrease in IOP and aqueous humor formation, and that isoproterenol caused a decrease in IOP comparable to that caused by epinephrine. Ross and Drance[36,37] also demonstrated that topical isoproterenol led to a dose-related decrease in IOP in patients with elevated IOP; however, this effect was lost after treatment over a two week period. Three of the four patients whose outflow facility was measured showed a decrease in facility. No increase in outflow facility was noted. This suggests that isoproterenol decreased aqueous formation.

Gaasterland et al.[38] investigated the effects of α- and β-adrenergic stimulation on aqueous dynamics in the eyes of healthy young men and demonstrated that isoproterenol applied topically caused a decrease in IOP and aqueous flow without affecting outflow facility. Norepinephrine had no effect on IOP or outflow facility but did increase

the flow rate of aqueous, although this increase was not statistically significant. Epinephrine caused decreases in IOP and aqueous humor flow and increased outflow facility; however, the increase in outflow facility was not statistically significant. Other studies[29,39] have supported the conclusions of Gaasterland concerning isoproterenol and epinephrine, but have demonstrated that norepinephrine does decrease IOP and increase outflow facility. Paterson and Paterson[40] have further demonstrated that salbutamol is effective in decreasing IOP in humans as well as in rabbits.[31]

Additional studies have demonstrated that epinephrine in humans does in fact increase outflow facility,[41-48] in agreement with the findings in monkeys by Barany[33] and Bill.[34] Krill et al.[43] reported an increase in outflow facility in only some of the patients in whom epinephrine caused a decrease in IOP. Kronfeld[44] reported an increase in outflow facility on the day of treatment with epinephrine and on the following day. He explained the observed decrease in IOP as being caused by the increase in outflow facility. Thomas and Epstein[49] also reported that topical epinephrine in humans caused an increase in facility of outflow. This increase in outflow facility was effectively blocked by timolol, a β-adrenergic antagonist. Sears[50,51] summarized these actions of epinephrine by defining three phases of action. During the early phase, occurring immediately after instillation of epinephrine, a decrease in IOP occurs which is secondary to both a decrease in aqueous

humor inflow (probably a result of decreased blood flow to the ciliary processes) and an increase in outflow facility; both effects are probably mediated by α-adrenergic receptor stimulation. During the intermediate phase, lasting for several hours after instillation, the increase in facility becomes the more important factor; this process is mediated in part by a β-adrenergic receptor. Later, over a period of days to months, there is a progressive increase in outflow facility.

Townsend and Brubaker[48] demonstrated with a fluorophotometric technique that epinephrine caused an early increase in aqueous humor formation. These photofluorometric results of aqueous humor formation differ significantly from previously studies. Weekers et al.[7] and Nagataki[52] demonstrated that epinephrine caused a decrease in the rate of loss of fluorescein from the anterior chamber, indicating a decrease in the rate of aqueous humor formation. A possible explanation for this apparent discrepancy is the time at which the measurements for determining aqueous humor formation were made. Weekers measured fluorescein disappearance 24 hours after instilling epinephrine into the eye, and estimated a decrease in aqueous humor formation of 30%. Nagataki found a suppression of aqueous formation of 20% at 10 hours. Townsend's data was taken earlier after epinephrine instillation. It has been reported that epinephrine can cause a transient, early increase in IOP which is followed by the sustained, delayed-onset decrease

in IOP.[30,31] The mechanism of this transient increase is unclear, but may be mediated by α-adrenergic stimulation, as this transient increase has also been noted to occur in eyes treated with norepinephrine and phenylephrine.[31] If this increase in IOP were caused by a transient increase in aqueous humor formation, it would explain the apparently contradictory findings of these reports, and still remain consistent with the other experimental findings concerning epinephrine.

The effects of adrenergic stimulation upon aqueous humor dynamics in the human eye as demonstrated by the previously discussed reports are summarized in Table 2.

The molecular basis for the β-adrenergic-mediated decrease in IOP has recently been an area of much research. It had earlier been shown that stimulation of β-adrenergic receptors results in activation of the enzyme adenyl cyclase, which catalyzes synthesis of adenosine 3',5'-monophosphate (cyclic-AMP), which, in turn, acts as a second messenger within the cell.[53] Waitzman and Woods[54] demonstrated the presence of a catecholamine-stimulated adenyl cyclase in the ciliary processes of rabbits. Further extensive work by Neufeld, Sears, and co-workers[55-58] demonstrated that epinephrine, norepinephrine, and isoproterenol, applied topically to rabbit eyes, all caused an increase in aqueous humor levels of cyclic-AMP, especially in the anterior chamber. A high correlation was found between the elevation of cyclic-AMP and the decrease in IOP. Also, when

cyclic-AMP and dibutyryl cyclic-AMP were perfused through the anterior chamber, increases in outflow facility similar to those seen with epinephrine were observed. Perfusion with isotonic saline or with adenosine 5'-monophosphate, an inactive metabolite of cyclic-AMP, failed to produce a change in outflow facility. The authors concluded that these findings supported the view that catecholamines activated adenyl cyclase by stimulating β-adrenergic receptors, increased intracellular cyclic-AMP, and increased outflow facility. The same experiments were duplicated by Neufeld[59] in the vervet monkey with similar results. Neufeld extended the study one step further by first injecting isoproterenol of epinephrine intracamerally, observing an increase in outflow facility, and then injecting cyclic-AMP and noting that no further increase in outflow facility occurred. This added further support to the conclusions of Sears and Neufeld.

Cholera toxin is known to stimulate adenyl cyclase, and has been useful in studying the action of adenyl cyclase in various tissues.[60-62] In studies by Gregory et al.[63] and Sears et al.,[65] cholera toxin was used to study what effect the stimulation of adenyl cyclase would have upon IOP and aqueous flow. Intravitreal injection of small doses (0.02 - 2.0 μg) of cholera toxin caused a remarkable decrease in ipsilateral ocular tension usually after 6 hours, without changing the IOP in the contralateral control eye. Intra-arterial infusion of 2 μg of cholera toxin via selective catheterization of the internal maxillary artery also led

to a decrease in IOP after 4 to 6 hours, and a 50% decrease in the rate of aqueous formation in the ipsilateral eyes. Intra-arterial infusion of saline had no effect on IOP. Ocular blood flow estimates made after infusion of cholera toxin indicated that blood flow to the anterior uvea increased as IOP fell, which ruled out the possibility that the decrease in aqueous humor formation was a result of decreased blood flow. Also, in vitro studies were conducted that demonstrated that incubation of ciliary processes with cholera toxin activated adenyl cyclase. A report published at the same time confirmed the findings of Gregory et al. that intravitreal cholera toxin results in a decrease in IOP.[64] The authors also reported an increase in outflow facility after intravitreal injection of cholera toxin, which supports earlier data that suggested that intracameral cyclic-AMP caused an increase in facility.[55,58]

Mishima et al.[66] have studied the anatomic location of the binding sites for cholera toxin in rabbit eyes using fluorescein-labeled and horseradish peroxidase-labeled cholera toxin. In vitro incubations of ciliary processes with fluorescein-labeled cholera toxin demonstrated multiple binding sites along the inner nonpigmented epithelial layer. Intra-arterial infusions of horseradish peroxidase-labeled cholera toxin via the internal maxillary artery demonstrated a dense reaction product located between the apical plasma membranes of the pigmented epithelium and the nonpigmented epithelium. These findings are consistent

with an epithelial binding site for cholera toxin and with biochemical studies supporting the premise that β-adrenergic agonists decrease IOP by a cyclic-AMP mediated decrease in aqueous humor secretion.

Radius and Langham[67] and Boas et al.[68] have questioned the importance of cyclic-AMP as a messenger involved in the catecholamine-induced decrease in IOP. Boas argued that in the case of epinephrine, aqueous humor cyclic-AMP is increased early after instillation; cyclic-AMP levels peak at 1 to 4 hours and decline to basal levels by 6 hours, but the decrease in IOP was not significant until 6 hours. Because of the discrepancies in the time association of elevated cyclic-AMP levels and decreased IOP, Boas concluded that the two were not related. He failed to take into account that the intracellular, not the aqueous humor, cyclic-AMP is the important factor, and the intracellular levels were not assessed. Also, as epinephrine may cause a transient increase in IOP,[30,31] it is possible that this effect masks the early action of a β-adrenergic mediated increase in cyclic-AMP which, in the absence of the early elevation in IOP (probably α-induced), would most likely have led to a decrease in IOP. It is interesting to note that pure β-adrenergic agonists cause a significant decrease in IOP at 1 to 2 hours,[29,31] which would correspond well to the time sequence of increased cyclic-AMP in the aqueous humor as reported by Boas. Boas also fails to consider that only minimal intracellular increases in cyclic-AMP

could be the trigger initiating a cascade of intracellular events leading to a delayed decrease in the IOP. Because of these considerations, the arguements of Boas are not credible.

Much work has also been done in attempting to localize anatomically the adrenergic receptors of the eye. Bhattacherjee[69] showed that uptake of topical and intracameral [^{3}H]norepinephrine in the ocular tissues of rabbits is complete within 2 hours. Extensive uptake in the epithelium of the ciliary processes was observed, as well as in the dilator muscle of the iris and the ciliary body, indicating rich adrenergic innervation of these tissues.

Neufeld and Page[70] identified α- and β-adrenergic receptors in preparations of rabbit iris-ciliary body by ligand binding techniques using [^{3}H]dihydroergocryptine and [^{3}H]dihydroalprenolol (specific for α- and β-adrenergic receptors respectively). Lahav, Dafna, and co-workers[71,72] injected 9-aminoacridinpropranolol, a fluorescent analog of propranolol into the right internal carotid artery of rabbits and into the tail vein of rats, and observed uptake of the analog in the ciliary body, iris, and episcleral tissue at the limbus of the rat. Almost no fluorescence was present in the ciliary muscle, while the sphincter of the iris exhibited diffuse fluorescence. In the rabbit, fluorescence was present over both epithelial layers of the ciliary epithelium. Only a few fluorescent cells were seen in the stroma of the ciliary processes. No fluores-

cence was seen in the trabecular meshwork.

Bromberg, Gregory, and co-workers[73,74] demonstrated that the highly specific β-adrenergic ligand [^{125}I]iodohydroxybenzylpindolol[75] ([^{125}I]HYP) binds to a single set of receptor sites in rabbit ciliary processes. Binding of [^{125}I]HYP was inhibited by β-adrenergic antagonists (l-alprenolol, d,l-propranolol, and l-timolol) and α- and β-adrenergic agonists (l-isoproterenol and l-norepinephrine), but not by an α-adrenergic antagonist (phentolamine). The authors concluded from these results that the [^{125}I]HYP binding site is a β-adrenergic receptor.

Studies of the adenyl cyclase of rabbit[74,76] and human[77] ciliary processes showed that catecholamines differed in their ability to activate the enzyme in the following order: isoproterenol > epinephrine > norepinephrine > phenylephrine. This sequence of sensitivity has been noted to be present in tissues with β_2-adrenergic receptors (vascular and bronchial smooth muscle), as opposed to β_1-adrenergic receptors (cardiac and adipose tissue, and small intestine).[78] Stimulation by isoproterenol[76,77] and epinephrine[74] was inhibited by low doses of β-adrenergic antagonists including timolol, but not by phenoxybenzamine[74] or phentolamine.[76] The selective β_2-adrenergic antagonists IPS 339 and H35/25 also inhibited isoproterenol-mediated stimulation of adenyl cyclase at low concentrations; whereas, selective β_1-adrenergic antagonists (atenolol and practolol) were less effective.[76,77] Furthermore, selective

β_2-adrenergic agonists (zinterol and OPC 2009) were much more effective in stimulating adenyl cyclase from rabbit ciliary processes than a selective β_1-adrenergic agonist (prenalterol).[76]

The most interesting aspect of the studies by Nathanson[76,79] was the assay of adenyl cyclase in dispersed cell fractions. It was shown that the basal and isoproterenol-stimulated adenyl cyclase specific activities were greater in the epithelial cell fraction, which contained both secretory and pigment cells, than in whole ciliary processes or the partially de-epithelialized vascular network.

These studies support, by a more direct approach, the conclusions drawn by using other techniques: that β_2-adrenergic receptors exist in the epithelial cells of the ciliary processes which, upon stimulation, activate adenylate cyclase.

Sears and Barany[13] published the first report of an adrenergic antagonist decreasing IOP. Since that report, much work has been done that demonstrates that adrenergic antagonists decrease IOP. The α-adrenergic antagonists prazosin, labetalol, thymoxamine, and phenoxybenzamine have all been shown to decrease IOP.[28,80,81] However, other studies have shown that thymoxamine and phenoxybenzamine may not lower IOP under all circumstances.[80,82,83] β-adrenergic antagonists have been more extensively studied than the α-adrenergic antagonists, and also are effective

in decreasing IOP. The non-selective β-adrenergic antagonist propranolol has been reported to decrease IOP in rabbits and humans by a number of investigators.[32,84-95] Authors have variously reported that propranolol decreases outflow facility,[89] increases outflow facility,[85] and decreases formation of aqueous humor.[85,89]

The non-selective β-adrenergic antagonists oxprenolol, timolol, pindolol, butridine, DCI, alprenolol, and bupranolol have also been shown to decrease IOP.[13,91,94-98] A study by Bonomi and Steindler[96] demonstrated a slight increase in outflow facility with pindolol, but the observed increase in facility was considered to be too small to account for the observed decrease in IOP.

Musini et al.[88] demonstrated that the non-selective β-adrenergic antagonists lignocaine and INPEA, devoid of local anesthetic properties, did not decrease IOP, while d,l-propranolol, with local anesthetic properties, did decrease IOP. They concluded that the local anesthetic properties of propranolol were responsible for its effect in decreasing IOP. However, Vale and Phillips[87] demonstrated that d-propranolol, which is identicle to d,l-propranolol with respect to its membrane-stabilizing and anesthetic effects, but only 1/60th as active in blocking β-adrenergic receptors,[99,100] was not effective in decreasing IOP. Therefore, the conclusions of Musini were not supported.

The selective β_1-adrenergic antagonists practolol, atenolol, metoprolol, sotalol, ICI 66082, and the selective

β_2-adrenergic antagonist IPS 339 have all decreased IOP in rabbits and humans.[92-94,101-105] Of these compounds, pindolol has only a slight membrane-stabilizing effect and no local anesthetic effect,[96] and practolol, timolol, sotalol, and atenolol are all devoid of both effects.[94,101,102] In spite of this, they are all capable of inducing significant decreases in IOP. In fact, Bonomi found that timolol and sotalol were the most active of the β-adrenergic antagonists tested in decreasing IOP.[94] These studies again demonstrated that it was not the local anesthetic properties of the β-adrenergic antagonists that were responsible for their ability to reduce IOP.

Of the adrenergic antagonists named, only timolol has been approved by the FDA for use in treating glaucoma, and therefore, timolol is the most studied ocular hypotensive antagonist. Timolol was first described by Hall[106] as a potent β-adrenergic antagonist, several times more active than propranolol, and devoid of sympathomimetic, local anesthetic,[107] and membrane-stabilizing effects.[94] Early studies of the effects of timolol in rabbits with α-chymotrypsin-induced elevated IOP and in rabbits with normal eyes by Vareilles et al.[98] demonstrated a gradual and sustained fall in IOP in rabbits with α-chymotrypsin-induced elevations in IOP after treatment with 0.5% and 1.5% timolol solutions. The maximum decrease occurred at 210 minutes after instillation. Only marginal decreases in IOP in normal rabbit eyes treated with timolol were noted.

Studies in 15 normal humans by Katz et al.[108] demonstrated that drops of 0.5%, 1.0%, and 1.5% timolol solution caused a significant decrease in IOP. Other studies have also demonstrated a decrease in IOP with topical timolol,[109-114] which is sustained over long periods with twice-daily dosage.[111] Decreases in IOP observed with timolol are additive with the decreases induced by both miotics and carbonic anhydrase inhibitors, indicating that timolol probably acts by some mechanism different than that by which both of these drugs act.[111,112]

Timolol has also been reported to cause an additional decrease in IOP over short periods when added to epinephrine therapy.[115-117] An additive decrease in IOP has also been noted in rabbits with normal IOP treated with timolol plus epinephrine or norepinephrine, but not timolol plus isoproterenol.[118] However, Thomas and Epstein[49] have noted that the additive effect of timolol and epinephrine in humans lasts for approximately 2 weeks, and at 8 weeks no significant additive decrease in IOP remains. Unfortunately, this study does not have a control group treated only with timolol or epinephrine for the 8 week duration of the study, and the findings are therefore difficult to interpret.

Zimmerman et al.,[109] Boger et al.,[113] and others[112,115,119-121] demonstrated that timolol lowered IOP without increasing outflow facility, even after long-term treatment. These investigators concluded that timolol acts by decreasing the formation of aqueous humor. In fluorophotometric

studies in humans, Coakes and Brubaker[122] and Yablonski et al.[123] demonstrated that the timolol-induced decrease in IOP was associated with a decrease in aqueous humor flow. Coakes measured a minimum decrease of 13%, a maximum of 48%, and a mean decrease of 34% in aqueous humor flow. Both reports concluded that the decrease in aqueous humor formation was most likely responsible for the decrease in IOP. This is also apparently the mechanism of timolol's ocular hypotensive activity in animals. Studies in cats by Helal et al.[124] demonstrated that intravenous timolol decreased IOP by 20% and aqueous humor formation by 62%, beginning at 45 to 60 minutes after injection. Liu et al.[125] also demonstrated that the decrease in aqueous humor formation was related to the concentration of timolol perfused through the anterior chamber of the cat eye.

The fact that both β-adrenergic agonists and antagonists act to decrease IOP by the same mechanism of decreasing aqueous humor formation is not consistent with the relationship of adrenergic agonist and antagonist seen in other tissues. Various explanations for this paradoxical situation have been proposed, one being that the β-adrenergic antagonists may cause a decrease in IOP in a way other than the blocking of the β-adrenergic receptors. This view has been dismissed by some[127] on the basis that d-propranolol, which has virtually no β-adrenergic blocking activity, also has no ability to decrease IOP.[87] Recent studies demonstrate that this is not the case for all d-

isomers of β-adrenergic antagonists. Liu and Chiou[128] have demonstrated that both l- and d-timolol are capable of decreasing aqueous humor formation without affecting the rate of aqueous outflow in the cat.

The work of Vareilles et al.[98] supports the theory that timolol decreases IOP by a mechanism other than β-adrenergic blockade. They demonstrated that timolol, in concentrations as low as 0.001%, applied topically, antagonized the inhibitory effect of isoproterenol on the water-load-induced elevation in IOP; this was felt to be due to timolol's β-adrenergic blocking activity. This study is very interesting because it vividly demonstrates that the β-antagonistic effects of timolol are present at low concentrations (0.001%), but that much greater doses are required to induce a decrease in IOP (0.5%).

Schmitt et al.[95] studied several β-adrenergic antagonists and demonstrated that topical application of 0.1% to 1.0% solutions of propranolol were capable of blunting the increase in IOP caused by water-loading in rabbits, while oxprenolol and practolol were not. 0.01% to 1.0% timolol solutions and 1.0% oxprenolol solutions blunted the isoproterenol-induced reduction of elevated IOP in water-loaded rabbits; whereas alprenolol, practolol, and propranolol at doses of 0.01% to 1.0% had no effect. Oxprenolol was capable of blocking the effect of l-isoproterenol on IOP in water-loaded rabbits, but had no effect on IOP by itself in the same animal model. On the other

hand, alprenolol and propranolol did not block the effect of isoproterenol on IOP, but did block the elevation of IOP induced by water-loading. They also demonstrated that 0.001% timolol, 0.01% oxprenolol, and 0.1% propranolol, but not alprenolol and practolol, reduced the increase in aqueous humor cyclic-AMP that had been induced by isoproterenol. Once again, concentrations of timolol, too small to decrease IOP, had been shown to effect β-adrenergic blockade. The authors concluded from these findings that there was little relationship between a β-adrenergic antagonist's ability to block β receptors and to decrease IOP.

Bergamini et al.[126] determined the I_{50} for several topically-applied β-adrenergic antagonists in rabbit eyes treated with epinephrine. The I_{50} is the concentration of antagonist needed to inhibit the IOP-lowering effect of topical epinephrine by 50%. The I_{50} of timolol was found to be 3×10^{-5}%. Other studies in rabbits have demonstrated that timolol is not capable of decreasing IOP at this low concentration.[95,98]

Washout studies with timolol[129] suggest that timolol applied topically to rabbit eyes inhibits the isoproterenol-mediated activation of adenyl cyclase for only 3 to 5 hours, even though the ability of timolol to decrease IOP is of much greater duration. If the ability of timolol to decrease IOP were secondary to its effect upon β-adrenergic receptors, it would be necessary for these receptors to be blocked throughout the duration of the effect, accord-

ing to the presently accepted model of the action of adrenergic antagonists. The fact that timolol does not remain at the receptors for an extended length of time argues against β-adrenergic blockade as the mechanism by which timolol decreases IOP.

These studies support the position that timolol reduces IOP by mechanisms other than β-adrenergic blockade. Several different authors have proposed this explanation based on different evidence.[74,124,128,130-133]

The purpose of this study was to estimate the concentration of timolol in the ciliary processes after topical administration of the drug in rabbit eyes. Two different concentrations of timolol were used; one capable of decreasing IOP, and the other incapable of decreasing IOP. If timolol were in fact decreasing IOP by β-adrenergic antagonism, the concentrations of timolol in the ciliary processes after topical instillation of both concentrations should be close to K_I for timolol binding to the β-adrenergic receptors in this tissue. If this were not the mechanism by which timolol is decreasing IOP, and if, as some data suggests, timolol lowers IOP at concentrations well above those required to achieve complete blockade of ciliary process β-adrenergic receptors, then the concentration of timolol in the ciliary processes should be much higher than K_I.

Preliminary studies to determine the concentrations of timolol to be used were conducted in rabbits with α-

chymotrypsin-induced increases in IOP. The effect on IOP of various concentrations of timolol in these animals was to serve as the basis for the selection of the concentrations of timolol to be used in determining the ciliary process concentration of timolol after topical application of the drug.

The concentration of timolol in the samples of ciliary process, iris, cornea, sclera, and conjunctiva was estimated by applying [^{3}H]timolol topically to rabbit eyes and determining the radioactivity in tissues removed from the eyes by liquid scintillation counting.

MATERIALS AND METHODS

MATERIALS

Drugs for in vivo studies

Timolol maleate as the solid, and [^{3}H]timolol (0.20 mg, 3.15 mCi in 5 ml ethanol) were generously donated by Merck, Sharp, and Dohme of Rahway, New Jersey. Both were the l-isomer. Pentobarbital sodium injection, USP (50 mg/ml, Nembutal) was obtained from Abbott Laboratories, North Chicago, Illinois. Proparacaine HCl 0.5% solution (Alcaine), and α-chymotrypsin (Zolyse, reconstituted with 5 ml of dilutent) were obtained from Alcon Laboratories, Fort Worth, Texas.

Instruments for eye examinations and IOP measurements

A Haag-Streit slit lamp was used for all biomicroscopic examinations. IOP measurements were made with an Alcon applanation pneumatonograph previously calibrated by intraocular manometry.

Materials for determination of concentration of timolol in ocular tissues

Collagenase (160 units/mg solid) was obtained from Sigma Chemical Co., St. Louis, Missouri. Protosol (0.5 M), Econofluor Scintillation Cocktail, and the tritium standard (2.42×10^6 dpm/ml) were obtained from New England Nuclear, Boston.

Radioactivity was determined in a Nuclear-Chicago Mark II Liquid Scintillation System.

RESPONSE OF IOP TO TOPICAL TIMOLOL

Preparation of animals

Twenty male New Zealand White rabbits, weighing 1.5 to 2.5 kg each were used in thses studies. The method of Sears and Sears,[134] with minor modifications, was used to induce an increase in IOP in the right eye of the rabbits.

The eyes of each rabbit were carefully examined with the slit lamp to ensure that the eyes were normal. The rabbits were placed under general anesthesia with Nembutal, 1.5 to 2.5 ml, injected into the marginal ear vein, and the anesthetized animal was laid on its left side. One drop of Alcaine was placed on the right eye and the eye proptosed. A 30-gauge needle was placed through the cornea into the anterior chamber to serve as a drain for aqueous humor and excess α-chymotrypsin during the injection. A second 30-gauge needle, attached to a syringe containing α-chymotrypsin, was inserted approximately 1 mm posterior to the limbus and the needle point situated in the posterior chamber. Care was taken to avoid damaging the lenticular capsule. 0.4 to 0.7 ml of α-chymotrypsin was injected into the posterior chamber, with care taken to distribute it as evenly as possible. Both needles were then removed, the eyelids closed to prevent drying of the eye, and the animal allowed to recover from anesthesia. The left eyes were left untouched. The animals were returned to their cages and allowed to feed and drink as desired. Six weeks elapsed before studies were conducted with the animals.

Treatment of animals with timolol

Animals in which the right eye had been treated with α-chymotrypsin were selected for these studies by the following criteria: 1) IOP in the right eye was significantly elevated over the IOP in the left eye. 2) The gross and biomicroscopic examination of the eye revealed no inflammation, flare, or clouding of the aqueous humor. 3) The animal had not been used in a previous study for at least seven days. The animals used in the studies were handled very gently to avoid exciting them.

The concentrations of timolol used in this study were 0.05%, 0.1%, and 0.5%. A 50 µl aliquot of the timolol solution to be tested was placed in each eye at time zero, and measurements of IOP made before, after, and at the time of installation of the timolol. The eyes were anesthetized with 1 drop of topical Alcaine prior to each measurement of IOP. After the IOP had been determined, the Alcaine was washed from the eye with normal saline to limit the duration of anesthesia.

Analysis of the data was made with a paired-difference t test, comparing the IOP of each eye with the zero-time IOP in the ipsilateral eye. The paired-difference t test was chosen because it is a much more sensitive test of the statistical significance than other tests utilizing only mean values and their standard deviations.

CONCENTRATION OF TIMOLOL IN OCULAR TISSUES

Preparation of [^{3}H]timolol

1.0 ml of [^{3}H]timolol (0.20mg, 3.15 mCi in 5 ml ethanol) was mixed with 5 mg of timolol maleate in 3 ml distilled water. This solution was lyophilized, and the resulting solid redissolved in sterile normal saline to give a 0.5% [^{3}H]timolol solution containing 1.7 x 10^9 dpm/ml. A 0.05% [^{3}H]timolol solution was prepared by making a 1:10 dilution of the 0.5% stock solution. The solutions were stored frozen.

Concentration of timolol in ocular tissues

Normal male New Zealand White rabbits weighing 1.5 to 2.5 kg with normal eyes by gross examination were used. The rabbits were placed in a plastic restraining box and allowed to become accustomed to the box. A 50 µl aliquot of 0.5% or 0.05% [^{3}H]timolol solution was layered over the left cornea, the lids held open for two minutes, and then the eye washed with 15 ml of normal saline. The same procedure was then repeated for the right eye, and the rabbit was left alone in the restaining box.

The rabbits were killed at 30 minutes after the installation of 0.5% timolol, and at 30 and 60 minutes after installation of 0.05% timolol by injecting 5ml Nembutal into the marginal ear vein. The right eye was enucleated and placed into 30 ml of Hank's balanced salt solution (BSS) on ice. The left eye was then enucleated, and samples of cornea, sclera, conjunctiva, iris, and ciliary processes removed. The

The sclera and conjunctiva samples were taken from the area of the insertion of the superior rectus muscle. The ciliary processes were isolated by quickly scraping them free from the iris. Tissue samples from the left eye were each kept in 1 ml of BSS or ice until the dissection of the right eye was completed. After the dissection of the left eye had been completed, the right eye was dissected in a similar manner, and each tissue sample placed in 1 ml of BSS or ice until weighed. The enucleation and dissection of both eyes was completed within 30 minutes of killing the animal.

The ciliary processes in BSS were centrifuged at 2550 rpm for 15 to 20 minutes, the supernatant discarded, and the net weight of the pellet determined. The other tissues were removed from the BSS and weighed at this time. 0.1 ml of collagenase (160 u/mg, 1mg/ml) was added to each tissue sample and the samples were allowed to stand a minimum of two hours at room temperature. 0.9 ml of Protosol was then added and the tissue samples were placed in a swirling water bath at 40°C until the tissues were completely dissolved. Collagenase was added prior to the addition of Protosol to the tissue samples because preliminary studies indicated that collagenase greatly facilitated dissolution of the tissue by Protosol. 3.0 ml of Econofluor was then added to each sample and the radioactivity determined with the Mark II liquid scintillation counter.

After the samples were counted, the counting efficiency

was determined by adding 10 μl of tritium (2.42×10^6 dpm/ml) to each sample as an internal standard and recounting the samples. The number of moles of timolol per kilogram of tissue was calculated from the amount of [^{3}H]timolol in the tissue samples (dpm), the specific activity of the [^{3}H]timolol, and the wet weight of the tissue samples.

RESULTS

RESPONSE OF IOP TO TOPICAL TIMOLOL

50 µl of 0.5% timolol produced significant decreases in IOP in both the α-chymotrypsin treated eyes and in normal eyes. 50 µl of 0.1% timolol produced occasional significant decreases in mean IOP in both eyes. A 50 µl aliquot of 0.05% timolol produced a significant decrease in IOP in only the α-chymotrypsin treated eyes. (Table 3)

CONCENTRATION OF TIMOLOL IN OCULAR TISSUES

The concentration of timolol in ocular tissues determined with [^{3}H]timolol are shown in Table 4. The concentrations of timolol were always higher in the right eye than in the left eye, with the single exception of the concentration of timolol in specimens of sclera after the application of 0.05% timolol. The differences between right and left samples of iris and cornea for both concentrations of timolol tested were significant at the $P < 0.05$ level, calculated by the paired-difference t test.

The concentration of timolol in ciliary processes, 30 minutes after installation of a 0.5% timolol solution, was 0.46 ± 0.22 µmoles timolol/kg tissue. The concentration of timolol in the ciliary processes 60 minutes after installation of a 0.05% solution was 0.21 ± 0.12 µmoles timolol/kg tissue. The concentration differences noted between 0.5% and 0.05% solutions at 30 and 60 minutes respectively were statistically significant for ciliary processes, iris, and cornea, but

not for conjunctiva or sclera. No apparent difference existed between the concentrations of timolol in the various ocular tissues determined at 30 and 60 minutes after installation of 0.05% timolol solution.

DISCUSSION

In this study, [^{3}H]timolol was applied topically to both eyes of normal rabbits, and the concentration of retained timolol in ocular tissues was determined. No attempt was made to determine whether the timolol was actively taken up by a receptor in the tissue, or if it had been retained by some other mechanism.

The studies of the response of IOP to topical timolol encouraged the use of 0.05% and 0.5% [^{3}H]timolol solutions to determine the concentration of timolol in ocular tissues; 0.05% timolol solution at 60 minutes because of its inability to decrease IOP in normal rabbit eyes, and 0.5% timolol solution at 30 minutes because of its ability to decrease IOP in both normal and α-chymotrypsin treated eyes. The early times after installation of the drug were chosen to minimize the amount of radioactive yet pharmacologically inactive metabolites of timolol present in the tissue.

Other studies have produced conflicting data about the ability of timolol to decrease IOP in normal rabbit eyes. Varielle et al.[98] reported a slight decrease in IOP after topical administration of 0.01%, 0.1%, and 1.0% timolol solutions. This response appeared to be independent of dose. On the other hand, Radius et al.[118] demonstrated in rabbits a decrease in IOP from 28.4 mmHg to 16.2 mmHg 15 minutes after topical application of 0.5% timolol solution. The present study demonstates a slight yet statistically signifcant

decrease in IOP in normal rabbit eyes using 50 µl of 0.5% topical timolol.

After application of 0.5% and 0.05% [^{3}H]timolol solutions, the concentration of timolol in iris and cornea in right eyes were significantly greater than the concentrations in left eyes. The concentration of timolol in the ciliary processes and conjunctiva was also higher in the right eyes than in the left, altough the differences were not statistically significant. This difference is most likely an artifact of the method used in enucleating and dissecting the eyes. The right eye was always enucleated first and immediately placed into 30 ml of BSS on ice: the left eye was then enucleated and dissected. Only after the dissection of the left eye had been completed was the right eye removed from the BSS and dissected. The dissection of both eyes was always completed in 30 minutes or less; it can therefore be estimated that the right eye was in the salt solution for about 15 minutes before the tissues were dissected free. During this time, uptake of timolol may have continued, resulting in higher concentrations of timolol in the tissues of the right eye.

Another important factor is that the tissues of the left eye were dissected free and then each specimen was placed in 1 ml of BSS. The retained timolol would thus have additional time to diffuse out of the tissue into the solution. These two factors, a result of the procedure employed in the dissection, are the most likely causes of the right-left differences in timolol concentration.

This factor - the allowing of any unbound retained timolol to diffuse freely out of the tissue into a timolol-free solution, also argues that the retained timolol is probably also bound in some way to the tissue. However, as has been previously emphasized, no studies of binding of timolol to actual receptor sites were performed.

It is also important to note that the procedure employed would lead to underestimation of the concentration of retained timolol in the tissues. Upon dissection, the tissues were placed in 1 ml of BSS, allowing retained timolol to leave the tissue and enter the solution. Wet samples for weighing may have contained excess water; this would have lead to overestimation of the tissue weight, thus decreasing the calculated timolol concentration per kilogram of tissue. Additional uptake in the right eye could possibly lead to overestimation of the retained timolol concentration in tissues; however, this overestimate in the right eye is averaged with the underestimate of timolol in the left eye. This should minimize the effect of overestimation of timolol concentration due to additional time for diffusion after the killing of the rabbit. Overall, these estimates of retained timolol concentration in ocular tissues in Table 4 are probably underestimates of the true tissue concentrations.

Bromberg et al.[73] and Gregory et al.[74] estimated K_I for binding of timolol to the β-adrenergic receptors in rabbit ciliary processes to be 0.6×10^{-9} M. Nathanson [76] estimated K_I to be 2.5×10^{-9} M.

The concentrations of timolol found in the ciliary processes after topical administration of 0.5% [^{3}H]timolol solution, 50 µl, a dose capable of decreasing IOP in normal eyes, was found in this study to be 4.6×10^{-7} moles/kg of tissue. (Table 4) Topical administration of 0.05% [^{3}H]timolol solution, which had not been capable of decreasing IOP in normal eyes, was 2.1×10^{-7} moles/kg of tissue. If the assumption is made that the tissue is 100% water, treatment with 0.5% and 0.05% timolol solutions resulted in 4.6×10^{-7} M and 2.1×10^{-7} M, respectively, in the ciliary processes. It should be noted that this assumption also results in an underestimate of the molar concentrations of timolol in tissue. These concentrations are, by a factor of 10^2 to 10^3, greater than the K_I for timolol estimated by Nathanson [76], Bromberg et al.[73], and Gregory et al.[74]. Therefore, it is evident that the concentration of timolol in the ciliary process far exceeds the amount necessary to saturate the β-adrenergic receptors in this tissue. It is significant that this is true for a dose of timolol (0.05%) which does not decrease IOP.

Using the equation of Cheng and Prusoff[135] in the form $I_{50} = \left(1 + \frac{[A]}{K_{act}}\right)$, where I_{50} is the concentration of timolol needed to inhibit 50% of the stimulation of adenyl cyclase by epinephrine in the rabbit, [A] is the concentration of circulating epinephrine in the rabbit (basal concentration 166 pg/ml, or 9.0×10^{-10} M)[136], K_{act} is the activation constant for

epinephrine in rabbit ciliary processes (3.6×10^{-7}M)[74], and K_I is the inhibition constant for timolol (1.2×10^{-9}M, the mean of values determined by Bromberg, Gregory, and Sears [73, 74], and Nathanson[76]). Using thses values, I_{50} for timolol is calculated to be 1.2×10^{-9} M. If Nathanson's value of K_{act} for epinephrine (2.35×10^{-6}M)[76] is used, I_{50} is also 1.2×10^{-9} M.

The rabbits used in these studies were obviously under stress, thus increasing the animals' serum epinephrine levels. Unfortunately, the authoer was unable to find values for circulating epinephrine in stressed rabbits. It can be seen by examining the equation for the computation of I_{50} that, for $[A] < K_{act}$, I_{50} approaches K_I. In order to increase I_{50} by a factor of 10, the concentration of circulating epinephrine would have to increase by a factor of 10^3. In humans with pheochromocytoma, a catacolamine-secreting tumor, the circulating epinephrine increases only by a factor of 10^2, even in pheochromocytoma that secretes epinephrine exclusively[137-140]. If an increase in circulating epinephrine is assumed to be the maximum increase experienced by the rabbits in this study, then the term $(1 + [A]/K_{act})$ increases only slightly, and the I_{50}, calculated using this higher serum epinephrine concentration, is equal to 1.5×10^{-9} M, almost identical to the I_{50} computed from basal epinephrine levels.

The concentrations of timolol found in the ciliary processes were greater by a factor of 10^2 than I_{50}; therefore,

it would be expected that the β-adrenergic receptors of the rabbit ciliary process are completely blocked after topical application of 50 µl of 0.5% and 0.05% timolol. If timolol's ability to decrease IOP were a result of β-adrenergic blockade, then both 0.5% and 0.05% timolol solutions should have caused a decrease in IOP; however, this was not the case. These findings add further support to the premise that timolol acts to decrease IOP by a mechanism other than β-adrenergic blockade.

This author was also very much interested in the work by Liu and Chiou[128] which suggests that l- and d-timolol are equally effective in decreasing the formation of aqueous humor. These findings are interesting because the d-isomers of β-adrenergic antagonists usually have little adrenergic blocking activity.[141] If this is the case for d-timolol - and there is no reson to suspect that it is not - then further research with d-timolol would provide a definitive answer to the question: is timolol's ability to decrease IOP the result of a mechanism other than β-adrenergic antagonism?

TABLE 1

Effects of adrenergic stimulation on aqueous humor dynamics in the rabbit

	IOP	Aqueous formation	Outflow facility
α stimulation	↓ (delayed onset)	(→)	↑
β stimulation	↓ (early onset)	(↓)	↑
β_1 stimulation	→		
β_2 stimulation	↓ (early onset)	(↓)	↑ *

↓ decrease ↑ increase → no change

Arrows in parenthesis designate conclusions inferred and not actually observed.

* Too small to alone account for decrease in IOP.

TABLE 2

Effects of adrenergic stimulation on aqueous humor dynamics in humans

	IOP	Aqueous formation	Outflow facility
α stimulation	↓	→	↑
α,β stimulation (epinephrine)	↓ (α,β effect)	↓ (β effect)	↑ (α effect)
β stimulation	↓	↓	→
β_2 stimulation	↓		

TABLE 3

Effect of topical application of 50 µl of timolol on IOP in rabbits

Group	Mean Intraocular Pressure (mm Hg ± S.D.) at Time after Instillation of Timolol							
	-60 min.	0 min.	30 min.	60 min.	120 min.	180 min.	240 min.	300 min.
Timolol 0.05% n = 10								
α-chymotrypsin	18 ± 2	19 ± 2		17 ± 2*	17 ± 2*	16 ± 2*	17 ± 2*	17 ± 2
normal	14 ± 1	15 ± 2		15 ± 1	14 ± 1	15 ± 1	16 ± 1	15 ± 1
Timolol 0.1% n = 4								
α-chymotrypsin	18 ± 3	18 ± 2		16 ± 2*	16 ± 2	17 ± 1	17 ± 1	18 ± 1
normal	14 ± 1	15 ± 1		13 ± 1*	14 ± 1	14 ± 1*	14 ± 1	15 ± 1
Timolol 0.5% n = 5								
α-chymotrypsin	18 ± 2	18 ± 2	17 ± 2*	17 ± 2	16 ± 1*	17 ± 2*		
normal	16 ± 1	16 ± 1	15 ± 0*	14 ± 1*	15 ± 1	16 ± 1		

* Significantly different ($p < 0.05$) from the pressure measured in the ipsilateral eye at time of instillation, by paired-difference t test.

TABLE 4

Concentration of timolol in ocular tissues of the rabbit

Group	Eye	n†	moles timolol/kg tissue (x 10^{-7})*				
			Ciliary Processes	Iris	Cornea	Conjunctiva	Sclera
Timolol 0.5% 30 min.‡	R eye	5	5.6 ± 2.5	5.0 ± 1.9	42.7 ± 19.0	12.2 ± 16.4	1.14 ± 1.07
	L eye	5	3.6 ± 1.3	2.0 ± 0.8	19.6 ± 9.2	5.5 ± 1.5	0.60 ± 0.33
	Combined	10	4.6 ± 2.2	3.5 ± 2.1	31.1 ± 18.6	8.8 ± 11.5	0.87 ± 0.80
Timolol 0.05% 60 min.‡	R eye	5	2.2 ± 1.4	2.3 ± 0.9	18.4 ± 7.2	2.2 ± 2.7	0.38 ± 0.28
	L eye	5	2.0 ± 1.1	1.4 ± 0.4	8.5 ± 3.6	1.3 ± 0.3	0.65 ± 0.84
	Combined	10	2.1 ± 1.2	1.8 ± 0.8	12.0 ± 6.3	1.7 ± 1.9	0.52 ± 0.61
Timolol 0.05% 30 min.‡	Combined	4	2.9 ± 0.7	1.9 ± 0.4	17.8 ± 5.5	1.5 ± 1.0	0.35 ± 0.29

* Expressed as mean ± S.D.

‡ Time between instillation of timolol and death of animal.

† Number of eyes.

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